

2P.29 Aqueous access channels in subunit a of sodium transporting F₀F₁-ATP synthase

Hiroki Sato¹, Noriyo Mitome¹,

Toshiharu Suzuki², Masasuke Yoshida^{1,2,3}

¹Chemical Resources Laboratory, Tokyo Institute of Technology, Japan

²ATP Synthesis Regulation Project ICORP,

Japan Science and Technology Corporation, Japan

³Faculty of Engineering, Kyoto Sangyo University, Japan

E-mail: nmitome@res.titech.ac.jp

F₀F₁-ATP synthase (F₀F₁) is the enzyme that synthesizes ATP from ADP and phosphoric acid by using the electrochemical potential gradient of the ion (H⁺ or Na⁺) between inside and outside of the membrane. The F₀ works as an ion channel in the membrane. The ion channels are thought to be composed of the acidic residue of c-ring located at the center of the membrane and two half-channels. It has been reported that both periplasmic and cytoplasmic sides of the H⁺ half-channels in H⁺-transporting F₀F₁ exist in a subunit. In contrast, it has been proposed that the periplasmic side of the Na⁺ half-channels in Na⁺-transporting F₀F₁ exists in a subunit and the cytoplasmic side exists in c-ring. However it is unclear where the Na⁺ half-channels are because the cytoplasmic side of the Na⁺ half-channels is not found from the structure of c-ring. In this study, we examined aqueous accessibility of a transmembrane helix of Na⁺-transporting F₀ by reactivity of cysteine substituted residue using a hybrid F₀F₁ (F₁ from thermophilic *Bacillus* PS3 and Na⁺-transporting F₀ from *Propionigenium modestum*). We predicted that both periplasmic and cytoplasmic sides of the Na⁺ half-channels in Na⁺-transporting F₀F₁ existed in a subunit as the H⁺ half-channels. Then, Cys residues were introduced into a subunit from a1211 to aV236 except aR226. After the modification of Cys of the mutant F₀F₁ with N-ethylmaleimide (NEM), the labeling yield of Cys by NEM and the ATP synthesis activity were examined. When Cys of G215C, K219C and N230C was labeled at 50% labeling yield, 40% of ATP synthesis activity was lost. These amino acid residues were accessible from outside of the membrane. In these mutants, ATP synthesis activity was inhibited because the modified Cys with NEM blocked Na⁺ transport. Therefore, it was suggested that G215C and K219C in the cytoplasmic side and N230C in the periplasmic side were the amino acid residues that formed the Na⁺ half-channels. It was suggested that both periplasmic and cytoplasmic sides of the Na⁺ half-channels in Na⁺-transporting F₀F₁ existed in a subunit as the H⁺ half-channels.

doi:[10.1016/j.bbabbio.2010.04.126](https://doi.org/10.1016/j.bbabbio.2010.04.126)

2P.30 On the rotary mechanism of F₁F₀-ATP synthases

Denys Pogoryelov¹, Alexander Krah², Julian Langer³,

José D. Faraldo-Gómez^{2,4}, Thomas Meier^{1,4}

¹Max Planck Institute of Biophysics, Department of Structural Biology, Frankfurt am Main, Germany

²Max Planck Institute of Biophysics,

Theoretical Molecular Biophysics Group, Frankfurt am Main, Germany

³Max Planck Institute of Biophysics,

Department of Molecular Membrane Biology, Frankfurt am Main, Germany

⁴Max Planck Institute of Biophysics, Cluster of Excellence Macromolecular Complexes, Frankfurt am Main, Germany

E-mail: Denys.Pogoryelov@mpibp-frankfurt.mpg.de

The mechanism of the F₁F₀ ATP synthase couples the downhill membrane translocation of H⁺ or Na⁺ to the rotation of an oligomeric ring of c-subunits (c-ring) in the F₀ motor. The torque is transduced into the F₁ motor, which causes sequential conformational changes in the catalytic centers, finally resulting in the

generation of ATP. The design of the c-ring rotor provides the ion binding specificity and contributes to the translocation of the ions through the membrane during enzyme operation. The crystal structure of the c₁₅ ring of the F₁F₀-ATP synthase from *Spirulina platensis* has been solved at 2.1 Å resolution [1]. The way the proton is bound to this c-ring proposes that all ion binding sites of the c-ring remain in the proton-locked conformation while exposed to the membrane, whereas exposure to a more hydrophilic environment can unlock the ion binding site and promote ion release. This model is supported by combined structural, biochemical and *in silico* generated data of the proton binding site.

Reference

[1] Pogoryelov D *et al.* (2009) *Nat. Struct. Mol. Biol.* **16**: 1068–1073.

doi:[10.1016/j.bbabbio.2010.04.127](https://doi.org/10.1016/j.bbabbio.2010.04.127)

2P.31 The crystal structure of bovine mitochondrial F₁-ATPase, grown in the presence of phosphonate reveals a new intermediate in the catalytic cycle

David M. Rees¹, Andrew G.W. Leslie², John E. Walker¹

¹The Medical Research Council Mitochondrial Biology Unit, Hills Road, Cambridge, CB2 2XY, UK

²The Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK

E-mail: walker@mrc-mbu.cam.ac.uk

The ground state structure of yeast F₁-ATPase has a phosphate ion bound in the β_E-subunit, whereas the ground state structure of bovine F₁-ATPase does not [1]. In order to try and gain structural information about the phosphate binding site in the bovine enzyme, crystals were grown in the presence of ADP, magnesium ions and the phosphate analogue, phosphonate. The structure solved to 2.5 Å resolution reveals surprisingly that ADP is bound in the nucleotide binding domains of all three catalytic subunits. However, the ADP molecule bound in the β_E-subunit does not have an associated magnesium ion, whereas a magnesium ion is bound in the β_{DP}- and β_{TP}-subunits, as in other structures of F₁-ATPase. In these respects, the structure is similar to that of yeast F₁-ATPase inhibited with residues 1–52 of yeast IF₁ [2]. This latter structure has been interpreted as representing a post-hydrolysis state in which the magnesium ion has been released from the catalytic site before ADP. No electron density was observed that could be interpreted as bound phosphonate, and its probable role appears to be to chelate divalent metal cations, including magnesium ions, rather than acting as a phosphate analogue.

References

[1] Bowler MW, Montgomery MG, Leslie AGW, Walker JE (2007) *J. Biol. Chem.* **282**: 14238–14242.

[2] Robinson GC, Montgomery MG, Mueller DM, Leslie AGW, Walker JE (2010) In preparation.

doi:[10.1016/j.bbabbio.2010.04.128](https://doi.org/10.1016/j.bbabbio.2010.04.128)

2P.32 The structure at 2.5 Å resolution of the complex of F₁-ATPase from *Saccharomyces cerevisiae* inhibited with yeast IF₁

Graham C. Robinson¹, Martin G. Montgomery¹, David M. Mueller², Andrew G.W. Leslie³, John E. Walker¹

¹MRC Mitochondrial Biology Unit, Cambridge, UK

²Rosalind Franklin University of Medicine and Science, Chicago, USA

³MRC Laboratory of Molecular Biology, Cambridge, UK

E-mail: walker@mrc-mbu.cam.ac.uk